Abstract No. malk46

X-ray Reflectivity Studies of Adsorbed Proteins on Langmuir Layers

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Beamline(s): X19C

The C2 domains of protein kinase C-α (PKC- α-C2) and cytosolic phospholipase ($cPLA_2$-C2) have been shown to interact with lipid in different modes (Medkova, M., and Cho, W. (1998) *J. Biol. Chem.* 273, 17544-17552; Bittova, L., Sumandea, M., Cho, W. (1999) *J. Biol. Chem.* 274, 9665-9672, etc.). Two protein-monolayer systems have been studied by X-ray reflectivity to determine how PKC- α-C2 and $cPLA_2$-C2 interact with the membrane in more detail. $cPLA_2$-C2 was studied with POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) monolayer supported on the buffered Ca^{2+} containing aqueous solution (20 mM HEPES, 0.1 mM $CaCl_2$, 0.1 M KCl, pH=7). PKC- α-C2 was studied with mixture of POPC and POPS (7:3 ratio) monolayer and the same buffer as in case of $cPLA_2$-C2. (POPS is 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoserine).

The specular reflectivity from each phospholipid monolayer was measured prior to injecting the protein solution into the subphase. The reflectivity can be explained by a two layer model roughened by capillary wave theory. The upper layer corresponds to the phospholipid acyl chains, while the lower layer (adjacent to the subphase) corresponds to the phospholipid headgroups. The fitting parameters include the thickness of the layers, the average electron densities of the layers and the interfacial roughness.

The effect of protein injection was different for each protein-phospholipid system studied. In case of $cPLA_2$-C2 with POPC reflectivity data showed the original phospholipid layer was modified and a three layer model was necessary to fit the data. The data analysis indicated that a third layer attached to the phospholipid headgroup region was formed. This additional layer corresponds to the proteins bound to the phospholipid layer. More detailed and extensive study of the change in the phospholipid monolayer is necessary to determine the extent of protein penetration into the lipid. Our preliminary measurements do not indicate adsorption of PKC- α-C2 to the POPC/POPS monolayer.

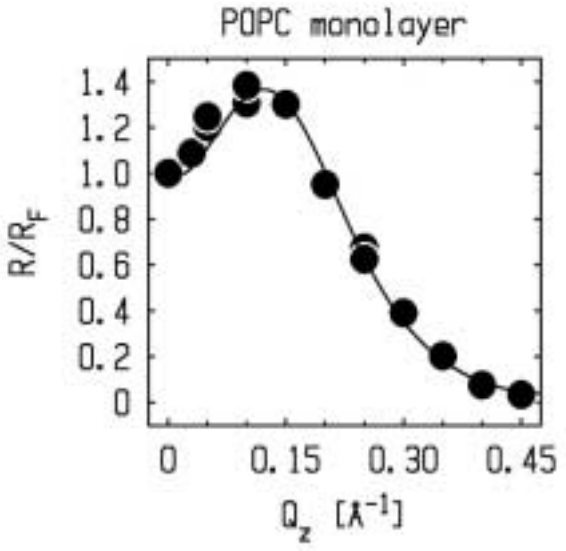

Figure 1 Normalized reflectivity R/R_F vs Q_z for POPC monolayer.

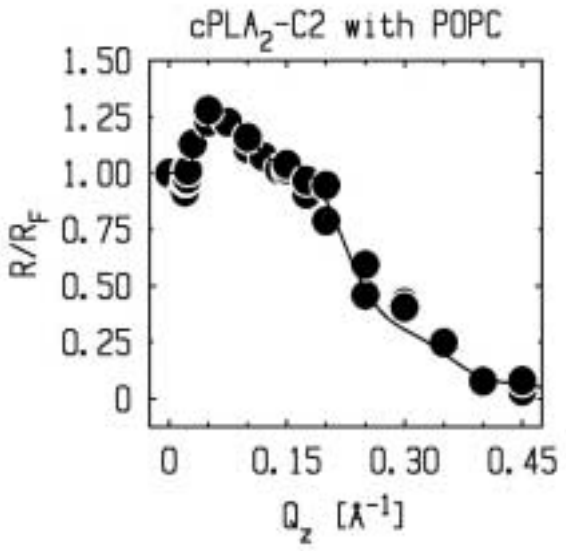

Figure 2 Normalized reflectivity R/R_F vs Q_z for POPC monolayer with $cPLA_2$-C2 adsorbed on it.